# Biosensors Research for Development of Innovative Monitoring Techniques That Support Exposure Assessment Related to the Superfund Program

**Kim R. Rogers**
U.S. EPA, ORD, NERL, HEASD, HERB

## 1 Introduction

One of the approaches for reducing uncertainties in the assessment of human exposure is to better characterize the hazardous wastes that contaminate our environment. A significant limitation to this approach, however, is that sampling and laboratory analysis of contaminated environmental and biological samples, can be slow and expensive; thus, limiting the number of samples that can be analyzed within time and budget constraints. In cases where indicator compounds can be identified, faster and more cost-effective field screening methods can increase the amount of information available concerning the location, source and concentration of these pollutants.

Among bioanalytical techniques reported for potential environmental monitoring applications, biosensors have recently generated a considerable amount of interest. Biosensors are analytical devices composed of a biological recognition element (e.g., enzymes, antibodies, nucleic acids or microorganisms) interfaced to a signal transducer (e.g., electrochemical, optical, or acoustic) which together relate the concentration of an analyte to a measurable signal. Because of the assay format versatility shown by biosensors, these devices may be able to overcome many of the limitations typical of biochemical assays used for environmental applications. Consequently, certain of these devices (when developed) will fill some of the gaps currently found among the field analytical technologies.

Although a wide range of biosensors for potential environmental applications have been reported, relatively few are likely to become commercially viable or show widespread use and acceptance in the highly competitive area of environmental field monitoring. Nevertheless, for certain niche applications, biosensor technology shows great promise. Consequently, the strategy used to select biosensor research projects under this task involves the choice of bioanalytical and sensor technologies that show the greatest potential to meet current and future analytical needs of the Agency. More specifically, these techniques are developed and characterized with respect to their feasibility for potential environmental applications.

## Staff

**Dr. Alma Apostol,** ***Senior Environmental Employment Program***
**James Cambrano,** ***Senior Environmental Employment Program***
**Dr. Niranjar Mishra,** ***National Research Council, Fellow***
**Dr. James Becker,** ***National Research Council, Senior Fellow***
**Rebecca Pearson,** ***UNLV, Masters Student***
**Steve Twomey,** ***UNLV, Student Volunteer***

## Selected References

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8 Wang, J., Lu, F., MacDonald, D., Ozsoz, M.E.S., Rogers, K.R. and Rotschler, C.L. (1998) Screen Printed Voltammetric Sensor for TNT. *Talanta* 46, 1405-1412.

## 2 Detection of Phenols Using a Field Portable Biosensor

Phenol and substituted phenols are widely used for industrial processes such as the manufacture of plastics, dyes, drugs, and antioxidants. These compounds also result as by-products from the paper pulp industry and breakdown products from organophosphorus and chlorinated phenoxyacetic acid pesticides. Because of their inherent toxicity, these compounds are of concern as pollutants in a variety of environmental matrices and are listed on the Priority Hazardous Compounds List from the Agency for Toxic Substances and Disease Registry.

Analytical methods for measuring phenols include colorimetry, gas chromatography, liquid chromatography, and capillary electrophoresis. Although these methods are sensitive and specific, they are also typically expensive and time-consuming. By contrast, for selected phenols, electrochemical biosensors are rapid and cost-effective as potential screening methods for these compounds.

*Figure 2.1*

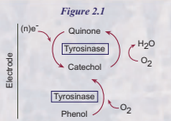



***Figure 2.1*** Biosensors that incorporate the enzyme tyrosinase have been shown to detect a number of monophenols and ortho catechols. This enzyme shows a hydroxylase activity by which phenols are hydroxylated to catechols using molecular oxygen and an oxidase activity that catalyzes the oxidation of catechols to quinones. When this enzyme is incorporated into carbon electrodes, the quinone product of phenol oxidation may be reduced electrochemically to the catechol at moderately negative potentials. Oxidation by the enzyme followed by reduction at the electrode results in cycling between the catechol and quinone and yields a catalytically amplified current. Detection of phenols using the enzyme electrode shows several advantages over both soluble enzyme assay methods and direct electrochemical oxidation. The signal amplification through cycling of the quinone product has been shown to increase the sensitivity of the enzyme electrode assay response by about 70 times.

***Figure 2.2***

***Figure 2.2*** Environmental Technologies Group Inc. (recipient of a recent EPA SBIR Phase II Award) is developing a field portable analytical system for measurement of phenols based on an enzyme biosensor. This technology is being evaluated by scientists in the biosensors research group at NERL-LV. The disposable sensor design eliminates the need to measure sample and handle reagents in the field. Operation of the sensor consists of several steps:

1. Add sample to the sample cup and close the cap (the cup meters the correct amount of sample).
2. Screw the cup into the sensor housing.
3. Flex the sensor to break the reagent ampule.
4. Shake the sensor to mix the sample and reagent.
5. Insert the sensor into the instrument.
6. Initiate the sample run (the instrument monitors the 5 min incubation and analysis period).

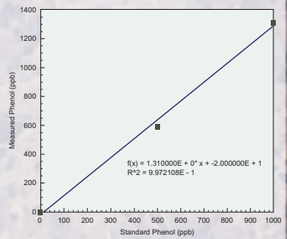



***Figure 2.3*** The instrument response is currently configured for an operating range of 200 to 1000 ppb phenol. The dynamic measurement range for the next prototype, however, will be 50 to 1000 ppb. The slope of the linear calibration plot varies in a predictable manner over the three month shelf life of the sensor and must be calibrated on a weekly basis. This biosensor method is currently being tested and evaluated using environmental samples contaminated with phenol.

## 3 Screening Assays for DNA Damage

Rapid and inexpensive indicator assays that can be used to screen for the genotoxicity of contaminated environmental samples and which can be related to a biological target (e.g., DNA) could be of significant benefit to the exposure assessment process. A variety of short term tests for genotoxicity/mutagenicity are currently being used to determine the extent of environmental hazards resulting from polluted water and sediments. Despite the description of short term, however, many of these assays are expensive to run, require sophisticated technical expertise, and are not well suited to be adapted to field applications. The focus of this project is the characterization of rapid, sensitive and inexpensive assays for detection of damage to surrogate sequences of DNA caused by environmental pollutants and stressors. These methods are expected to provide the Agency with rapid, sensitive, and simple techniques that can be used among a panel of methods to determine the genotoxic potential of polluted samples.

***Figure 3.1*** The assay being developed for this project primarily detects single strand breaks in target DNA -- although this technique may also be sensitive to double strand breaks, adduct formation, and base losses. The concept for this assay is as follows: under certain conditions (i.e., high temperature or alkaline pH), double stranded DNA will unwind into single strands. Because temperature-induced unwinding (denaturation) occurs either more rapidly or under milder conditions if the DNA backbone has been broken along one of the strands, it can be used as an indicator of chemically-induced single strand breaks.

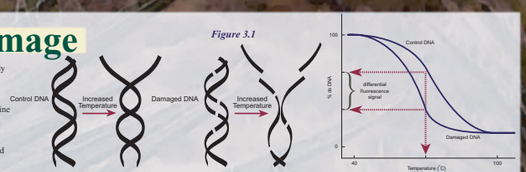



***Figure 3.1***

***Figure 3.2*** The degree of DNA denaturation is determined using the double strand sensitive dye PicoGreen. This dye indicator dramatically increases its fluorescence in the presence of double strand (as opposed to single strand) DNA.

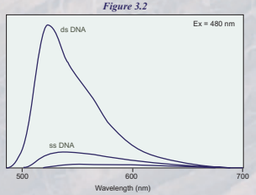



***Figure 3.2***

***Figure 3.3*** The relative fluorescence response of this assay for calf thymus DNA is indicative of radiation-induced damage as a function of dose. The fluorescence response increased rapidly with increasing doses of radiation and began to saturate at dose levels above 10 cGy. The inset shows the low dose response. A student's t test analysis of mean responses indicated that doses of 0.8 cGy and above yielded responses that were significantly different from the controls ($P = 0.02$).

These results demonstrate a rapid technique for detection of radiation-induced DNA damage using temperature rather than pH to differentially unwind oxidatively damaged DNA. Because the rapid and reversible control of temperature is relatively easy to accomplish from an engineering standpoint, this technique lends itself to the development of automated screening techniques. In addition to simple operation using standard laboratory instrumentation, this assay is particularly sensitive to radiation-induced DNA damage at levels as low as 0.8 cGy. Future work will involve integration of this technique into an automated analysis system to screen for chemically-induced damage to target DNA and correlation of this assay to (more expensive and time-consuming) classical assays for genotoxicity/mutagenicity and DNA damage.

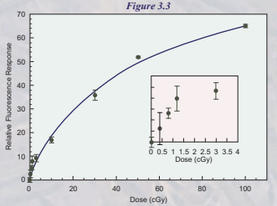



***Figure 3.3***

## 4 Detection of TNT Using Screen-Printed Electrodes

Production, open-burn/detonation testing, and storage of explosive compounds (primarily 2,4,6-trinitrotoluene, TNT) has resulted in significant contamination of a number of munitions facilities throughout the U.S. Due to the mutagenic, toxic, and persistent nature of these compounds, their leaching into ground water and accumulation in the food chain has generated interest in the characterization and cleanup of contaminated sites as well as concern for human and ecosystem exposure. In response to the need for rapid and cost-effective field analytical methods for detection of these compounds, rapid screening assays based on chemical, immunochemical and electrochemical techniques have been recently reported. Although each of these techniques can perform relatively rapid analyses, each method shows limitations in areas such as instrument cost, consumable cost, selectivity or sensitivity. Among these methods, one of the promising techniques uses square wave voltammetry in combination with disposable screen printed electrodes. This project will demonstrate improved detection limits for this technique.

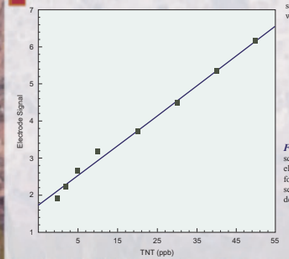



***Figure 4.2*** The coupling of solid phase extraction and square wave voltammetry with the use of disposable electrodes results in a dynamic assay range of 2 ppb to 5 ppm for TNT. The simplicity, rapidity, cost-effectiveness and sensitivity of this assay make it an excellent candidate for development as a field analytical method.

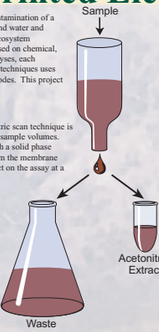
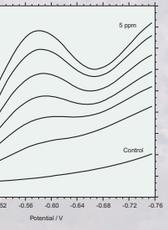



***Figure 4.1***

***Figure 4.1*** The square wave voltammetric scan technique is used to measure TNT in as little as 50 μL sample volumes. This electrochemical assay is coupled with a solid phase extraction. The explosive is extracted from the membrane using acetonitrile, which shows little effect on the assay at a final concentration of 10%.




## 5 Screening for OPs Using Acetylcholinesterase

Organophosphorus and carbamate insecticides are widely used in agriculture, commercial and residential settings. Due to their high toxicity in mammalian systems, they also pose a potential hazard to humans and ecosystems. Insecticides of these two chemical classes exert their toxic effects by means of inhibition of the enzyme acetylcholinesterase (AChE) in the peripheral and central nervous system. Consequently, to screen environmental media for the potential to result in human exposure to these classes of compounds, there is considerable value in monitoring the effect on the target enzyme (AChE).

Screening assays using surrogate AChE are typically sensitive, reliable and extensively reported in the literature. Several versions of this assay are also commercially available. There are, however, certain limitations for the application of these assays to environmental monitoring. These limitations include the variability of assay responses to various OP and carbamate insecticides (particularly the parent compounds that tend to show lower sensitivity than their oxidative metabolites). Work on this project will focus on (i) increasing the sensitivity of this assay to parent compounds by chemical or biochemical oxidation, and (ii) calibrating variable compound sensitivity in terms of paraoxon equivalents.

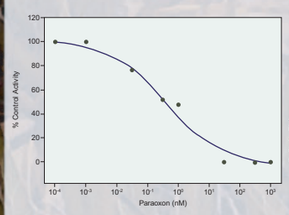



***Figure 5.2*** The assay is simple, inexpensive and sensitive. The enzyme can be stabilized in the plate wells for up to a week at room temperature using gelatin and trehalose.

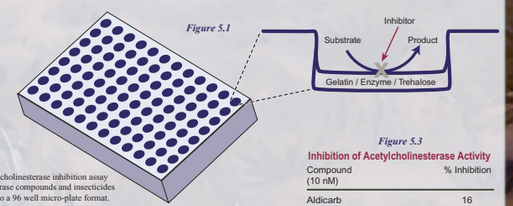



***Figure 5.1***

***Figure 5.1*** The cholinesterase inhibition assay for anti-cholinesterase compounds and insecticides has been adapted to a 96 well micro-plate format.

***Figure 5.3*** The assay has been demonstrated using several organophosphorus insecticides, carbamate insecticides and pharmaceuticals. Future directions will include the use of chemical and biochemical oxidation to increase the sensitivity of the assay for insecticides, standardization of the assay in terms of paraoxon equivalents and application of the assay for use with environmental samples.

***Figure 5.3***

**Inhibition of Acetylcholinesterase Activity**

| Compound (10 nM) | % Inhibition |
|---|---|
| Aldicarb | 16 |
| Methomyl | 4 |
| Carbaryl | 46 |
| Carbofuran | 42 |
| Dichlorvos | 94 |
| Eserine | 81 |
| Pyridostigmine | 62 |
| Naled | 95 |